HONG KONG
wonders never cease

Workshop on Assisted Reproduction Technology

27 October - 1 November, 1997

Queen Mary Hospital, Hong Kong

organized by

Department of Obstetrics & Gynaecology

& Centre of Human Reproduction,

University of Hong Kong

110 years

Faculty of Medicine

WELCOME MESSAGE

On behalf of the Department of Obstetrics and Gynaecology and the Centre of Human Reproduction, University of Hong Kong, I would like to welcome all the participants to our first Assisted Reproduction Technology Workshop. In the recent few years, there has been an increase in demand for training in assisted reproduction technology in China and other countries in this part of the world. By organizing this workshop, we want to share our experience with the participants. We have also invited a number of international experts to help us in our workshop. We hope that you can acquire enough knowledge to start or improve your own programme. I also hope that you can take this opportunity to see our city. I wish all of you a memorable stay in Hong Kong.

Professor P.C. Ho
Head, Department of Obstetrics and Gynaecology,
Director, Centre of Human Reproduction,
University of Hong Kong

Workshop on Assisted Reproduction Technology

Monday 27 October

0830 Registration (6/F, Professorial Block, Queen Mary Hospital)
0850 Opening address by Prof. S.P. Chow
0900 Physiology of folliculogenesis and ovulation (Dr. W.S. O)
0945 Physiology of fertilization and implantation (Prof. R.G. Edwards)
1030 Discussion (Moderator : Dr. W.Yeung)
1045 Tea break
1115 *In vitro* culture of embryo (Prof. M.H. Johnson)
1200 Discussion (Moderator : Dr. W. Yeung)
1215 Lunch
1330 Genetic control of development (Prof. R.G. Edwards)
1415 General principles and setting up of an ART program (Dr. W.W.K. So)
1500 Discussion (Moderator : Prof. P.C. Ho)
1515 Tea break
1545 Hormonal stimulation regimes (Prof. P.C. Ho)
1630 Factors affecting ovarian response to controlled ovarian hyperstimulation and clinical pregnancy rate in an IVF-ET program (Prof. L.Z. Zhang)
1715 Discussion (Moderator : Dr. W.W.K. So)
1730 End

Tuesday 28 October

0800 Practical / Tutorial / Case study / Live demonstration
1300 Lunch
1400 Patient selection and counselling (Dr. E.H.Y. Ng)
1430 Ultrasound in ART (Dr. W.W.K. So)
1500 Discussion (Moderator : Prof. P.C. Ho)
1515 Tea break
1545 Laboratory requirement and protocol (Dr. E.Y.L. Lau)
1645 Discussion (Moderator : Dr. W. Yeung)
1700 End
1900 Dinner

Wednesday 29 October

0800 Practical / Tutorial / Case study / Live demonstration
1300 Lunch
1400 Sperm assessment and preparation (Dr. W.S.B. Yeung)
1430 Gamete and embryo freezing (Dr. E.Y. L. Lau)
1500 Discussion (Moderator : Dr. E.Y.L.Lau)
1515 Tea break
1545 Oocyte and embryo donation (Prof. G.L. Zhuang)
1630 Alternative methods of conception (Dr. E.H.Y. Ng)
1715 Discussion (Moderator : Dr. W.W.K. So)
1730 End

Thursday 30 October

0800 Practical / Tutorial / Case study / Live demonstration
1300 Lunch
1400 Medical treatment of male infertility (Prof. A. Kung)
1430 Surgical treatment of male infertility (Dr. P.C. Tam)
1500 Discussion (Moderator : Dr. E. Ng)
1515 Tea break
1545 The use of electroejaculation in the treatment of anejaculatory males (Prof. S.W.J. Seager)
1630 Micromanipulation in ART (Dr. W.S.B. Yeung)
1715 Discussion ((Moderator : Dr. E.Y.L. Lau)
1730 End

Friday 31 October

0800 Practical / Tutorial / Case study / Live demonstration
1300 Lunch
1400 Current and future advances in ART (Prof. P.C. Ho)
1445 Role of nurses in ART (Prof. P.L. Sullivan)
1530 Discussion (Moderator : Dr. E. Ng)
1545 Tea break
1615 Ethical and legal issues of ART (Dr. A. Liu)
1700 Discussion (Moderator : Dr. W.W.K. So)
1715 End

Saturday 1 November

0800 Practical / Tutorial/ Case study / Live demonstration
1230 Awarding of certificates

Closing ceremony

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Physiology of folliculogenesis and ovulation

Dr. Wai-sum O
Department of Anatomy
The University of Hong Kong
Hong Kong China.

Growth of the follicle

The initiation of follicular growth occurs when a primordial follicle, which is formed during embryonic life, is activated by a yet unknown mechanism and enters the pool of proliferating follicles. The first event of follicular growth is the transformation of the single layer of epithelial cell to cuboidal granulosa cells characteristic of the primary follicle. Concomitant with this process is the initiation of mitotic activity of the granulosa cells that leads to an increased number of concentric granulosa cell layers that surrounds the oocyte, thus leading to a progressive increase in size of the preantral follicle. Although the control of the growth of the preantral follicles is poorly understood, the pattern of gonadotrophin secretion seen during the follicular phase is not essential for the initiation and maintenance of preantral follicle growth.

The largest size of preantral follicles seen in human ovary has six to seven layers of granulosa cells. After the preantral stage, under the appropriate hormonal support, the follicle may develop to the antral stage. The antrum in the follicle separates the granulosa cells into two distinct populations, the mural granulosa cells which surround the basement membrane along the perimeter of the follicle and the cumulus granulosa cells that surround the oocyte. The maturation of antral follicles to the preovulatory stage is absolutely dependent on the presence of the pituitary gland. Although the exact stage of follicular growth at which gonadotrophin become required is uncertain, it is likely that gonadotrophins become required concurrent with or shortly after the initiation of antrum formation.

Preantral follicles possess follicle stimulating hormone (FSH) receptors but not luteinizing hormone (LH) receptors. Thus the major hormone responsible for the transition of the preantral follicle into a preovulatory follicle is FSH. The major effects of FSH on follicular differentiation occur in the granulosa cell and include the induction of cell surface receptor for LH, the induction of the aromatase enzyme responsible for the conversion of androgens into oestrogens and the induction of the enzyme responsible for converting cholesterol into progesterone. Whereas, the actions of FSH on the maturing follicle are confined exclusively to the granulosa cells, the actions of LH on the maturing follicle are initially confined to the theca layer of small antral follicles because these are the only cells that possess LH receptors. Following stimulation by FSH, the granulosa cells acquire LH receptors by Day 9-10 of follicular phase and become responsive to LH.

The length of time required for the maturation of a follicle from the time it leaves the pool of primordial follicles until it ovulates has been estimated to take as long as 150 days. Accordingly, it is highly likely that a follicle that ovulates in a give cycle actually begins to mature before the follicular phase in which it will ultimately ovulated. These follicles are recruited at ≤ 4 mm diameter. Early in follicular phase prior to selection of follicles, plasma oestrogen concentrations are low while plasma FSH concentrations are slightly elevated. As the oestrogen concentration rises during the mid- through late- follicular phase, plasma FSH concentrations fall. These relationship could constitute a sensitive feedback system: once a follicle is stimulated by FSH and develops sufficient aromatase to elevate peripheral oestrogen concentration, FSH secretion would be inhibited, thus curtailing the growth of less mature follicles. As maturing follicles gains increased sensitivity to FSH during its course of maturation, concentrations of FSH that were unable to initiate growth of less mature follicles are effective to maintain oestrogen secretion and the growth of the mature follicles. This explains why maturing follicle continues to develop in the presence of FSH concentrations which are insufficient to maintain the growth of less mature follicles.

Preovulatory changes and ovulation

Preovulatory changes in antral follicles follow a gradual rise in the plasma level of gonadotrophic hormones. The steroid hormone output of the preovulatory follicles is enhanced to cause a LH surge. Oocytes that acquire the competence to resume meiosis in response to the LH surge is mediated through the granulosa cells. In the antral follicle, inhibitory levels of cAMP generated by the granulosa/cumulus compartment are continuously transferred to the oocyte to maintain it in meiotic arrest. In response to the preovulatory LH surge, the communication in the follicle is interrupted. As the flow of cAMP from the follicle to the oocyte decreases below the threshold levels required for maintenance of meiotic arrest, the oocyte resumes meiotic maturation. Germinal vesicle breakdown is the first morphological manifestation of the resumption of the first meiotic division.

During oocyte maturation, meiosis is resumed and proceeds through the remainder of the first meiotic division to result in expulsion of the first polar body. The oocyte proceeds to metaphase of the second meiotic division when maturation is arrested and ovulation occurs. Meiosis will not be completed until the oocyte is fertilized by a sperm.

The mechanism of ovulation has been the subject of debate. Factors that are important in bringing about follicular rupture include the action of hormones, prostaglandins, neural and enzymatic factors, osmotic pressure, permeability of blood vessels and contractility of the follicle wall.

Following ovulation, the ruptured follicle undergoes important changes which convert it into the corpus luteum. The transformation of the ruptured follicle is programmed before ovulation under the influence of LH. The lesser secondary follicles that fail to ovulate undergo atresia.

In vitro culture of the conceptus

Professor Martin H. Johnson
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Downing Street
Cambridge United Kingdom

The ability to fertilize oocytes *in vitro* and to culture them to the blastocyst stage offers great opportunities for scientific and medical investigation and advance, but also presents several dangers. Culture conditions *in vitro* do not mimic conditions *in vivo*, which change with both the time of the menstrual cycle and with progress of the developing oocyte/conceptus from follicle to oviduct to uterus. *In-vitro* culture conditions have been arrived at empirically by observation of the effects of manipulating culture conditions on both the development *in vitro* and the potential for further development on transfer to the uterus. For most species, it is not possible to culture conceptuses from fertilization to a viable blastocyst with full developmental potential. Even for the mouse, a species on which most progress has been made, the cumulative success rate for fertilizing oocytes *in vitro*, culturing to the 2-cell stage and, after transfer, gaining healthy live young is of the order of only 10-20%. For humans, it is difficult to estimate the equivalent rate but it is likely to be of the same order. These outcome rates seem at first sight to be disappointing, but to be certain of their meaning, it is essential to know what the equivalent *in vivo* outcome rates are. For both mouse and human, only estimates can be made based on very limited data. It seems likely that the number of young mice born per oocyte ovulated *in vivo* is considerably more than 10-20%. For humans, however, the only available data suggest that *in vivo* rates may not be too different from *in vitro* rates, but the data *in vivo* are not robust.

Possible sources of pregnancy loss during *in vitro* fertilization are poor quality oocytes or spermatozoa, suboptimal culture conditions *in vitro*, poor transfer technique, inadequate endocrine priming or response of the endometrium and later pregnancy loss. At the moment, we cannot be sure of the relative roles of each of these factors. The role of *in vitro* conditions will be focused on and three main points considered.

First, there is a developmental cell cycle which is characteristic for the species, at which developmental arrest or delay is most likely to occur e.g. 2-cell stage in the mouse, 8 cell stage in the human and 16-cell stage in the cow. This cell cycle is also the point at which major transcriptional activation of the conceptus' own genome occurs. However, arrest or delay is not simply a consequence of the failure to activate transcription.

Second, there is now considerable evidence that conditions which favour free oxygen radical production impair development, whilst those that prevent free radical production or ameliorate their effects promote development. In the mouse conceptus, there is a burst of free radical production at the 2-cell stage but only after culture *in vitro*.

Third, the early human conceptus shows a high incidence of chromosomal and/or nuclear abnormalities in many of its constituent cells. These abnormalities may be evident early (2-and 4-cell stages), and their incidence may be influenced by the culture medium used as well as by the quality of oocyte from which the conceptus has developed. The abnormalities may pose problems for the viability and developmental potential of the conceptus, as well as for preimplantation diagnosis of genetics disease.

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General Principles and the Setting up of an ART Program

Dr. So Wai Ki William
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Queen Mary Hospital
Hong Kong China

Introduction

Since the birth of Louise Brown in the summer of 1978, *in vitro* fertilization and embryo transfer (IVF-ET) and variants of the technique, collectively referred to as assisted reproductive technologies (ART), have become the established treatment for subfertility secondary to tubo-peritoneal disease, pelvic endometriosis, male factor, immunological factor or unexplained aetiology. Their development depend on the interaction of multiple disciplines including reproductive endocrinology, embryology, molecular biology, ultrasonography, urology and clinical psychology. Fundamental to these procedures are the 'production' and, in many instances, the recovery of multiple mature (and fertilizable) oocytes from the ovary with minimal trauma and expense to the patient.

Selection and Preparation of Patients

Any couple who presents with subfertility must be thoroughly evaluated as to the cause(s) of their predicament. Assisted reproductive technologies can then be offered whenever it is appropriate. Dr. Ernest Ng will discuss these issues in greater detail.

Controlled Ovarian Stimulation

Although the first IVF-ET success was from an unstimulated cycle and there are centres that still offer natural cycle IVF-ET, most centres favour controlled ovarian stimulation in view of the higher success rate associated with this method. Stimulation protocols used will be covered by Professor Ho Pak Chung. It will suffice to reiterate the main objective is to maximize the number of mature oocytes and to minimize the asynchrony between the remaining cohort of intermediate-sized follicles. A protocol that results in 6 to 10 pre-implantation embryos is considered to be optimal.

Monitoring Ovarian Response and Uterine Receptivity

Ultrasonography is the method used, with or without concomitant hormonal assays (serum E_2), to monitor the ovarian response to stimulation and to time the administration of human chorionic gonadotrophin (hCG). The role of serum E_2 will be discussed. Recent interest have been directed to the assessment of uterine receptivity as an additional parameter in the evaluation.

Collection of Oocytes for In Vitro Fertilization

Laparoscopy was the original method used to retrieve (mature) oocytes. The ultrasound-guided approach has now largely superseded laparoscopic retrieval. It can be performed in an ambulatory setting with conscious sedation resulting in a quicker recovery, less cost and minimal trauma to the patient. The requirements varied from an out-patient operating facility with full surgical capabilities to a clean (non-sterile) procedure/treatment room. The former allows for the use of light anæsthesia and/or conversion to laparoscopy if needed. With the latter arrangement, facilities for resuscitation and emergency laparotomy should be easily accessible. Irrespective of the type of facility where the oocytes are collected, it should be as close as practicable to the laboratory where fertilization is to take place.

There is no consensus on which type of needle is better for oocyte retrieval. Both single- and double-lumen needles are being used. The latter is usually preferred when follicles are routinely flushed with culture medium. There is again disagreement on whether flushing the follicle will improve the oocyte retrieval rate.

Laboratory Facilities

Specialized laboratory facilities for *semen preparation* and *embryo culture* need to be established. The requirements and protocol are described by Dr. Estella Lau. It is desirable that centres can provide a private and comfortable room where semen samples can be produced. The source of gametes and any embryos subsequently produced should be accurately recorded and labeled in a manner that is not susceptible to unauthorized and undetectable alteration. Blood products with which gametes and embryos may come into contact must be pre-tested for hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV). Precautions should be taken to avoid other potential sources of contamination including radiation, chemical and atmospheric agents during the handling and storage of gametes and embryos.

Embryo Transfer

Pre-implantation embryos are routinely replaced via the transcervical route 48 to 72 hours after oocyte retrieval. Some countries e.g. the United Kingdom, restrict the number of oocytes or embryos replaced to not more than three in any one cycle. A variety of transfer catheters are commercially available. They are either end-open or side-open with or without a stiff outer sheath. The end-open catheter has a theoretical disadvantage of its end being plugged by mucus or tissue. The stiff outer sheath facilitates negotiation of a tortuous cervical canal as well as avoids excessive manoeuver with the embryos in situ. The choice of catheter is based on personal experience and preference as no system has been shown to be superior.

Patients have the misconception that prolonged bed-rest is required after embryo transfer to avoid expulsion of these embryos from the uterus. Comparative studies have not demonstrated any difference in the success rate irrespective of the time the patient remains motionless after the transfer. Nonetheless, many centres allow their

patients to stay in bed from 1 to 3 hours immediately after the procedure more for psychological reasons than anything else.

Counseling

In addition to general information-giving including the nature and risks of the ART procedures (to the woman and any resulting child), the limitations and possible outcomes of the treatment, counseling should be made available. This includes *implications, support and therapeutic* counseling.

Refinements to ART

The last two decades have seen a number of refinements aimed at improving the success of assisted reproductive technologies. The use of GnRH agonists to down-regulate the pituitary have resulted in less number of cancellations, more optimal timing of hCG administration and the recovery of more mature oocytes. Ultrasound monitoring of ovarian response and ultrasound-guided oocyte retrieval has greatly simplified the procedure and reduced the cost. Cryopreservation of excess or 'spare' embryos has reduced the need for repeated stimulation cycles and improved the overall success rate. Fertilization can now be achieved with a single sperm via intracytoplasmic sperm injection (ICSI). Sperms can be retrieved either microsurgically with the open method or by percutaneous aspiration from the epididymis or directly from the testes in men with obstructive and non-obstructive azoospermia respectively.

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Patient Selection and Counselling

Dr. Ernest H.Y. NG
Assistant Professor
Department of Obstetrics and Gynaecology
The University of Hong Kong
Hong Kong China

Assisted reproductive technologies have been used to treat various causes of subfertility. More powerful techniques e.g. intracytoplasmic sperm injection are now available to assist fertilization. It is important to diagnose the full extent of a couple's problem before appropriate treatment can be offered. Both partners should be seen (preferably together) and investigated accordingly as multiple factors may be present and may involve both of them. Standard investigations of subfertility include semen analysis, ovulation monitoring, tubal patency, cervical mucus/sperm interface and implantation site. After the diagnostic work-up, the next step is to estimate the baseline prognosis without therapy and then determine whether treatment can increase the likelihood of pregnancy. The decision on treatment may involve choosing from several options and for each option the couple should be counselled about the chance of success, possible complications and costs of the treatment before a particular treatment is started.

I. *Male partner*

Semen analysis is essential. Semen samples are usually produced by masturbation after a period of 2 to 3 days of sexual abstinence. Several samples may be required because of wide fluctuation over time. The samples should be delivered to the laboratory within a short time and kept at body temperature during transport to minimize "cold-shock". Standard semen analysis includes count, percentage of motile and normal forms. The WHO criteria are widely adopted (Volume > 2 ml, Concentration > 20 x 106/ml, Forward motility > 50% and Morphology > 30%). It is important to appreciate that it provides only weak prediction of fertility because it tells nothing about sperm function which is the key to conception. The use of sperm function tests e.g. acrosome reaction, strict morphology, sperm-zona binding etc. is discussed. Other tests that can be performed include culture, hormone assay(FSH/LH), karotyping and testicular biopsy.

II. *Female partner*

A. *Ovulation* -- The woman can be asked to document the basal temperature every day and ovulation can be indicated by a biphasic pattern (basal body temperature chart or BBT chart). It is safe to assume that women with normal regular menstrual cycles in the range of 23 to 35 days ovulate normally in most cycles. This can be confirmed by serum progesterone level during the mid-luteal phase i.e. one week before the expected menstrual period. In women with

irregular cycles, serum prolactin, thyroxine, FSH and LH levels should be checked to find out the cause of anovulation. Pelvic ultrasound is useful in the diagnosis of polycystic ovaries.

B. *Tubal patency*--Hysterosalpingogram is a radiological investigation. The (radio-opaque) dye is injected through the cervix into the uterus, the tubes and the peritoneal cavity. Tubal damage may be indicated by the absence of dye spillage into the peritoneal cavity or the absent filling of the tubes. Diagnostic laparoscopy is done under general anaesthesia and it allows not only the tubal status but also the presence of pelvic adhesions and endometriosis. Chlamydia antibody level has been used to predict the extent of tubal damage.

III. ***Sperm-mucus interaction***

This can be assessed by the post-coital test, which involves examining a small sample of cervical mucus under a microscope for the presence of swimming sperm about 12 hours after intercourse. The mucus should be collected at the peri-ovulatory time, ideally one or two days before ovulation. It is then most copious, stretchy, slippery and looks clear. The problems and limitation of postcoital test will be discussed.

IV. ***Implantation site:***

The congenital anomalies and the presence of polyps in the uterine cavity can be detected by hysterosalpingogram and diagnostic hysteroscopy. For patients with recurrent failure of implantation, Doppler ultrasound scanning has been used to assess the state of uterine blood flow.

The indications for in vitro fertilization and embryo transfer (IVF-ET) are inoperable tubal damage, poor sperm quality, long-standing endometriosis and unexplained infertility. Assisted fertilization technique is required if less than 100,000 motile sperms are recovered after sperm preparation or less than 20% fertilization rate in a previous standard IVF cycle. Surgical retrieval of sperm may be needed in cases of azoospermia: micro-epididymal sperm aspiration (*MESA*) for obstructive causes and testicular sperm extraction (*TESE*) for testicular failure.

Subfertile patients undergoing treatment are under great psychological stress and disturbances. Counselling is important to provide accurate information and empathetic support to the couple. It is especially invaluable when subfertile women experience pregnancy loss or discontinue therapy without success.

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Hormone Stimulation Regimens

Professor HO Pak Chung
Chair in Obstetrics and Gynaecology
The University of Hong Kong
Hong Kong China

It has been shown by various studies that the success rate of assisted reproduction increases with the increase in the number of oocytes or embryos replaced. Therefore, although some assisted reproduction programs used natural unstimulated cycles, in most programs, ovarian stimulation is used to obtain more oocytes.

Use of Gonadotrophin-releasing hormone agonists (GnRHa)

When spontaneous luteininzing hormone (LH) surge occurs during ovarian stimulation, the oocyte retrieval has to be performed sometimes at inconvenient times. There is also some suggestive evidence that high LH levels during the follicular phase or premature luteinization is associated with an unfavourable outcome. Therefore, most assisted reproduction programs, GnRH agonists are used to reduce the chance of spontaneous LH surge during ovarian stimulation. There are two commonly used GnRH agonist protocols:

1. *Long protocol* -- The pituitary is suppressed by the GnRHa before gonadotrophins are used to stimulate the ovaries. The GnRHa may be started in the mid-luteal phase of the cycle preceding the treatment cycle or it may be started in the follicular phase. The suppression may be confirmed by the absence of follicles > 10 mm on ultrasound or a low serum estradiol (E_2) or LH level.
2. *Short protocol* -- Gonadotrophins are started at the same time as the GnRHa at the beginning of the treatment cycle. The advantage is that the flare effect of the GnRHa is used to stimulate the ovaries. The dosage of gonadotrophins may be decreased. However, about 6% of patients still develop LH surge. The ultra-short protocol os used less often.

Use of clomiphene citrate

Clomiphene citrate has been used in the past alone or together with human menopausal gonadotrophin (hMG) to stimulate the ovaries. With the increase in popularity of the use of GnRHa, this is less commonly used.

Use of gonadotrophins

There are several preparations of gonadotrophins: (a) hMG, (b) urinary follicle stimulating hormone (uFSH), (c) highly purified form of urinary FSH (uFSH-HP), and (d) recombinant FSH (rFSH). A recent meta-analysis (Daya *et al*, 1995) suggested that the use of uFSH may be associated with a better pregnancy rate than hMG. However, in this meta-analysis, studies using GnRHa are analyzed together with studies without using GnRHa. The three studies without GnRHa contributed

heavily to the increased odds ratio. Therefore, it is uncertain whether uFSH is an advantage when GnRHa are used. The main advantage of uFSH-HP is that it can be given subcutaneously by the patients themselves. There is some recent evidence suggesting that the use of rFSH is also associated with a better pregnancy rate when compared with cycles using uFSH and hMG (Out *et al*, 1997).

There are a large variety of ovarian stimulation protocols. In most programs, a daily dose of 150 IU or 225 IU is used. In our program, we started the stimulation with 300 IU hMG daily for the first two days. The dose is decreased to 150 IU daily from the third day onwards. The ovarian response is monitored with serum E_2 or transvaginal ultrsound. In recent years, an increasing number of programs use only pelvic ultrasound for monitoring. When there are more than three follicles with a mean diameter of at least 16 mm and the leading follicle is > 18 mm, the gonadotrophin is stopped and human chorionic gonadotrophin (hCG) is given. Oocyte retrieval is performed 34 - 38 hours after hCG injection.

Luteal phase support

Luteal phase support is necessary when GnRHa are used for pituitary suppression. Even without the use of GnRHa, many programs also provide luteal support. The luteal phase can be supported by means of hCG injections, progesterone suppositories or injections. When there is risk of ovarian hyperstimulation syndrome, hCG should not be given for luteal support. Progesterone suppositories or injections should be used instead.

Ovarian Hyperstimulation Syndrome (OHSS)

The main risk of ovarian stimulation is the development of ovarian hyperstimulation syndrome. The risk can be reduced by careful monitoring of the patients. When there is high risk for the development of OHSS, all embryos may be cryopreserved instead of being replaced. This is because if the woman conceives after embryo transfer, OHSS may be more severe and prolonged. The GnRHa may also be continued during the luteal phase to suppress the LH levels.

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Factors Affecting Clinical Pregnancy Rate in an IVF-ET Program

Li-Zhu Zhang, Zhi-Xin Wei, Xiu-Zhu Tian, Pin Liu, Jie Wu
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The IVF-ET program was started in our department in 1987, and the clinical pregnancy rate has improved through the years. The indication for IVF-ET has been bilateral tubal blockage and/or pelvic adhesions. The following data give an overview of our work in the past few years.

1. IVF-ET Cycles

Year	Number of cycles
1987-1994	757
1995	232
1996	198
Total	1187

2. Clinical Pregnancy Rate

Time period	Pregnancy Rate	Numbers
1987-June 1988 (1.5 years)	6.4%	5/78
July 1988-1991 (3 years)	16.3%	49/301
1992-1993 (2 years)	18.1%	43/237
1994	25.5%	36/141
1995	22.4%	52/232
1996	25.3%	50/198

3. Take Home Baby Rate

Year	Numbers	Pregnancy Rate
1992	11/105	10.48%
1993	15/132	11.37%
1994	28/141	19.86%
1995	44/232	18.97%

4. First Case in Mainland China

Test tube baby delivered on March 10, 1988.

GIFT baby delivered on March 18, 1988.

Triplet test tube baby delivered on August 26, 1989.

Oocyte donation test tube baby delivered on June 12, 1992.

Frozen-thawed embryo test tube baby delivered on February 6, 1995.

Surrogacy test tube baby delivered on September 22, 1996.

Material and methods

The following investigation was done to study the various factors which may affect the clinical pregnancy rate. The study consisted of three parts :

1. Clinical analysis of 559 IVF-ET cycles from July, 1992 to November, 1995.
2. Study of 104 IVF-ET cycles from September, 1994 to September, 1995, to see the different responses to the same controlled ovarian hyperstimulation (COH) regimen, chiefly FSH, hMG/hCG.
3. Analysis of IVF-ET cycles from November, 1995 to November, 1996, to study the hormonal changes in the luteal phase after embryo transfer (62 cycles), and some immunological factors (150 cycles) in relation to the clinical outcome.

Statistical analysis :

SPSS-PC+V3.0	X^2 Analysis of Single Factor Variants
EGRET V2.0	Single Factor Logistic Regression Analysis

Results

Part I

Table I-1 Patients from Different Areas in China and Clinical Pregnancy Rate

Area	IVF-ET cycles		Clinical Pregnancy	
	No.	%	No.	%
Beijing	128	22.3	23	18.0
Beijing + Hebe	276	49.4	49	17.8
Others	284	50.6	72	25.4
Total	559	100.0	121	21.6

Table I-2 Primary Sterility, Secondary Sterility and Clinical Pregnancy Rate

	IVF-ET cycles		Clinical pregnancy	
	No.	%	No.	%
Primary sterility	341	61.0	70	20.5
Secondary sterility	218	39.0	51	23.4
Total	559	100.0	121	21.6

$P = 0.4855$

Table I-3 History of Intrauterine Pregnancy and Clinical Pregnancy Rate

History	Cycle		Clinical pregnancy	
	No.	%	No.	%
Intrauterine pregnancy	103	47.3	22	21.4
Ectopic	115	52.7	29	25.2
Total	218	100.0	51	23.4

Table I-4 History of Early Intrauterine Pregnancy, Midterm and Term Pregnancy and Clinical Pregnancy Rate

History	Cycle (Secondary Sterility)		Clinical pregnancy	
	No.	%	No.	%
Artificial abortion	76	34.9	13	17.1
Midterm termination	4	1.8	3	75.0
Tubal ligation	9	4.1	2	22.2
Request another pregnancy	14	6.4	4	28.6
Total	103	47.3	22	21.4

$P = 0.057$

Table I-5 Tuberculosis as a Cause of Tubal Blockage and Clinical Pregnancy Rate

	Tuberculous	Tubal blockage	Clinical pregnancy	
	No.	%	No.	%
Primary sterility	129	23.0	28	21.7
Secondary sterility (after ectopic)	30	5.3	8	26.7
Total	159	28.4 (159/559)	36	22.6

Table I-6 Age and Pregnancy Rate

Age	Clinical Pregnancy		No pregnancy	Total
	No.	%	No.	
30	32	23.4	105	137
31 -34	63	22.1	222	285
35 - 39	24	19.5	99	123
40	2	14.4	12	14
Total	121	21.6	438	559

$P = 0.7389$

There is a tendency for the clinical pregnancy rate to drop as the age of the patients increases, although there is no statistical difference, probably because the number in each group varies too much.

Table I-7 Number of Embryos Transferred and Clinical Pregnancy Rate

Embryos transferred	Clinical Pregnancy		No pregnancy	
	No.	%	No.	Total
1	4	6.3	64	64
2	15	19.7	61	76
3	12	15.6	65	77
4	25	20.9	100	125
5	50	32.5	104	154
6	15	23.8	48	63
Total	121	21.6	438	559

$P < 0.001$

The highest clinical pregnancy rate (32.5%) occurred when 5 embryos were transferred. The number of embryos transferred is usually limited to 4, but it also depends upon the type of patients and the morphology of the embryos.

The CES/No. of embryos was determined in order to assess more accurately the quality of embryos.

The following results lead us to think that more oocytes should be retrieved to get more embryos and better embryos in order to increase the pregnancy rate.

Table I-8 Cumulative Embryo Score (CES) and Clinical Pregnancy Rate

CES	Clinical Pregnancy		No pregnancy	Total
	No.		No.	No.
> 40	85	28.3	215	300
< 40	36	13.9	223	259
Total	121	21.6	438	559

The CES is calculated according to Edwards' original criteria.

Table I-9 CES/No. of Embryos Transferred and Clinical Pregnancy Rate

CES/No. of	Clinical Pregnancy		No pregnancy	Total
embryos transferred	No.	%	No.	No.
≥ 11	75	26.2	211	286
< 11	46	16.8	227	273
Total	121	21.6	438	559

$P = 0.0097$

Part II

There are different ovarian responses to the same COH regimen-chiefly FSH, hMG/hCG.

Table II-1 Ovarian Response to COH

Type of Response	No. of follicles*	Cycle number	%
Low	< 3	8	7.7
Moderate	3 -14	78	74.9
High	> 14	18	17.4

* *Diameter > 10 mm*

Table II-2 Follicle Number, Size, Basal Serum E_2 Levels and Types of Ovarian Response

Type of Response	Size of follicle (cm)	Follicle Number	Basal serum E_2 (pmol/L)
Low	0.73 ± 0.70	5 ± 3	91.38 ± 71.22
Moderate	0.55 ± 0.47	9 ± 4	117.60 ± 60.10
High	0.42 ± 0.35	17 ± 11	110.33 ± 61.26
χ^2 analysis	$P < 0.05$	$P < 0.0001$	$P > 0.05$

Table II-3 Basic Serum FSH, LH Levels and Ovarian Response

Type of Response	FSH (IU/L)	LH (IU/L)
Low	15.5 ± 6.3	8.3 ± 4.8
Moderate	12.2 ± 3.6	6.3 ± 3.7
High	10.8 ± 2.3	9.7 ± 9.4
χ^2 analysis	$P < 0.05$	$P < 0.05$

Table II-4 Changes of Serum E2 Levels and Ovarian Response

Type of Response	Follicular Phase serum E_2 levels (pmol/L)		
	Day 3	Day 8	Before hCG
Low	91.38 ± 71.22	513.5 ± 17.7	1542.5 ± 1302.6
Moderate	117.6 ± 60.10	1786.7 ± 1277.7	5837.2 ± 3584.4
High	110.3 ± 61.26	5754.8 ± 2440.4	19140.8 ± 10717.5
χ^2 analysis	$P > 0.05$	$P < 0.05$	$P < 0.05$

Table II-5 Cycle Day 8 Serum FSH, LH Levels and Ovarian Response

Type of Response	FSH (IU/L)	LH (IU/L)
Low	32.7 ± 16.4	8.5 ± 6.3
Moderate	22.4 ± 7.7	6.6 ± 3.0
High	25.6 ± 6.6	19.4 ± 7.1
χ^2 analysis	$P > 0.05$	$P < 0.05$

Table II-6 Age and Basal FSH Level

Age	Basal FSH > 15 IU/L
< 35	18.4%
> 35	39.3%

Table II-7 Apoptosis of Oocytes Cultured in Vitro in Different Age Groups

Age group	No. of oocytes	Apoptosis		Time of apoptosis
		No.	%	Hrs.
1. 21 - 30	72	10	13.9^{Δ}	$39.4 \pm 4.2^{*}$
2. 31 - 40	69	26	37.7	30.3 ± 3.2
3. 41 - 50	65	40	61.5^{Δ}	$24.5 \pm 4.2^{**}$

$^{\Delta}$ $P < 0.01$, * $P < 0.005$, ** $P < 0.001$ as compared to group 2

Table II-8 Age and Ovarian Response

Type of Response	Age								Total
	25 -29		30 - 34		35 - 39		40 - 44		
	No.	%	No.	%	No.	%	No.	%	No.
Low	1	3.7	1	2.0	5	20.0	1	33.3	8
Moderate	19	70.4	38	77.6	19	76.0	2	66.7	78
High	7	25.9	10	20. 4	1	4.0	0	-	18
Total	27	-	49	-	25	-	3	-	104

Table II-9 Type of Ovarian Response and Pregnancy Rate

Type of response	Follicle number	Clinical pregnancy rate
Moderate	3 - 13	30.6% (22/72)
Low	< 3	25% (2/8)
High	> 13	0% (0/24)
Total		23.1% (24/104)

Therefore, high ovarian response with many oocytes retrieved does not mean a high pregnancy rate. High ovarian response associated with high serum E_2 levels and high LH levels may have some adverse effects on the quality of oocytes and on hormonal environment not favourable to embryo implantation.

Part III

Table III-1 Hormonal Changes in the Luteal Phase in Natural and IVF-ET Cycles

Cycle	Number	Serum E_2 (pmol/L)	Serum P (nmol/L)	P/E_2	PRL (ng/ml)
Natural *	63	378.7 ± 66.1	38.7 ± 17.9	118.1 ± 50.2	19.7 ± 14 3
IVF-ET *	62	5321.6 ± 1548.9	1341.5 ± 841.5	290.9 ± 102.4	53.4 ± 27.1

* $P < 0.05$

Table III-2 Luteal Phase Hormonal Changes and IVF Outcome

Group	Cycle	E_2 (pmol/L)*	P (nmol/L)	P/E_2*	PRL (ng/ml)*
Clinical pregnancy	15	4885.5 ± 1269.0	1679.4 ± 1076.5	373.0 ± 152.5	66.4 ± 26.8
No pregnancy	36	6091.2 ± 1522.1	1203.9 ± 818.7	227 4 ± 116.7	49.1 ± 24.1

* $P < 0.05$

Table III-3 Intramuscular Progesterone Supplement in the Luteal Phase and Changes in Hormonal Levels

Group	Cycle	E_2 (pmol/L)*	P (nmol/L)	P/E_2*	PRL (ng/ml)*
No supplement	6	4195.3 ± 928.1	43.7 ± 119.7	167.6 ± 70.7	35.1 ± 18.7
P 30 mg	45	5152.9 ± 1855.1	1399.9 ± 903.4	312.2 ± 101.7	54.0 ± 26.9
P 50-100 mg	9	5486.4 ± 2101.7	1279.8 ± 784.1	288.1 ± 131.4	52.6 ± 34.6

* $P < 0.05$

Table III-4 Number of Oocytes Retrieved, Changes of Hormonal Levels in the Luteal Phase and Clinical Pregnancy Rate

Group	Cycle	E_2 (pmol/L)*	P (nmol/L)	P/E_2*	PRL (ng/ml)*	Clinical Pregnancy (%)*
Oocytes <3	11	3537.0 ± 484.3	664.0 ± 35.4	215 ± 54	26.9 ± 12.6	18.2
Oocytes 3-8	28	5149.4 ± 1047.2	1561.1 ± 1155.0	349 ± 74	61.3 ± 22.3	42.8
Oocytes 8	12	6325.5 ± 1187.1	1017.0 ± 787.0	185 ± 44	52.1 ± 20.0	20.0

*$P < 0.05$

Further analysis demonstrates that when P/E_2 ratio is >300, the clinical pregnancy rate is high [54.4% (6/11)], and also when PRL is in the range between 60-100 ng/ml the pregnancy rate is 45.5% (10/22).

Table III-5 Serum Antisperm Antibodies in Infertile Women and Clinical Outcome

Group	Cycle	Oocytes no.	Cleavage %*	Clinical pregnancy %	Abortion %
ASA (+)	44	11.3 ± 6.5	64.2 ± 32.1	31.5	10.0
ASA (-)	106	10.2 ± 7.5	84.8 ± 18.7	21.4	7.7

* $p < 0.05$

Table III-6 Serum Anticardiolipin Antibodies (ACA) in Infertile Women and IVF-ET outcome

	ACA (+)	ACA (-)
Cycle	21	129
Oocyte retrieved (No.)	11.2 ± 10.4	11.3 ± 6.7
Cleavage Rate (%)	75.3 ± 26.6	78.5 ± 18.6
No. of embryos transferred	4.1 ± 1.0	4.3 ± 1.0
Clinical pregnancy no.	2	34
Clinical pregnancy %*	9.5	26.3

* $P < 0.05$

Table III-7 Relationship between Anticardiolipin Antibodies (ACA) and Clinical Pregnancy, Biochemical Pregnancy and No Pregnancy

Group (pregnancy)	Cycle	No. of embryos transferred	CES	Endometrial thickness (cm)	ACA (+) No.	ACA (+) %*
Clinical	36	5.0 ± 1.0	57.8 ± 22.6	1.1 ± 0.0	2	5.5
Biochemical	15	4.4 ± 1.4	59.6 ± 26.9	1.1 ± 0.1	3	20.0
No pregnancy	99	4.4 ± 1.4	57.8 ± 25.2	1.0 ± 0.1	16	16.2

* $P < 0.05$

Table III-5 shows that although the cleavage rate is affected by antisperm antibodies in the serum of the female, the clinical pregnancy rate is not ($P > 0.05$). However, the presence of anticardiolipin antibodies in the women is not favourable to embryo implantation and its early development (Tables III-6 and -7).

Discussion

The clinical outcome of IVF-ET is affected by many factors. The pregnancy success rate is related to the number and quality of embryos transferred. However, the number of embryos transferred should be limited to avoid high order multiple pregnancy, and the quality of the embryos could not be judged only by the morphology. There is much research to be done in this respect.

Ovarian response to the same COH regimen is different in different patients, and may be predictable to some extent. It is related to the basal (on the third day of the cycle) follicle number, more follicles mean higher response, large size of basal follicles means low response. In patients with a high basal FSH level (> 15 IU/L) the response is low. The basal FSH level increases with age of the patient.

High response associated with high E_2 levels and high LH levels on the eighth day of the stimulation cycle may exert an adverse effect on the quality of embryos, on embryo implantation and its early development. Clinical pregnancy rate is high [30.6% (22/76)] in the moderate response group.

Increased E_2 levels with lowered P/E_2 ratio in the luteal phase after ET may have an adverse effect on embryo implantation. This further supports our previous view point that high response may not give a favourable hormonal environment for embryo implantation.

The immunological mechanism of embryo implantation is complicated and remains controversial. Our work shows that antisperm antibodies present in the female serum may decrease the cleavage rate *in vitro* but not the pregnancy rate, and therefore IVF-ET may still be the treatment of choice for such patients. Anticardiolipin antibodies should be determined in the female patients in a planned IVF-ET program, and anticoagulants prescribed to improve the pregnancy rate.

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Ultrasonography in Assisted Reproduction

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Introduction

Ultrasonography has played a pivotal role in the development and simplification of assisted reproductive technology (ART) treatments. In the early days it was used concomitantly with serum E_2 levels, to monitor ovarian response and to initiate urine collection for detection of the LH surge. It has now become the sole method in many centres around the world for monitoring multiple folliculogenesis and in timing the administration of hCG. Ultrasound-guided oocyte retrieval has become the gold standard for oocyte collection and has to a large extent abolished the need for a pre-treatment laparoscopic assessment. Ultrasonography has been applied in the management of difficult embryo transfers, in guiding transcervical tubal cannulations and in the management of ART-associated complications. It has an important role in early pregnancy assessment following ART and plays an integral part in multifetal pregnancy reduction (MFPR) procedures. Transvaginal ultrasonography together with colour Doppler studies have been increasingly evaluated as a non-invasive method of assessing uterine receptivity and in predicting implantation and/or pregnancy outcome. On the horizon are the possible use of ultrasound-derived indices of follicular blood flow to predict oocyte recovery and pre-implantation embryo quality and the place of three-dimensional ultrasonography in assessing follicular volume.

Pre-treatment evaluation

The improved resolution achieved with transvaginal ultrasonography and the recent development of (saline-infusion) sonohysterography have enabled uterine abnormalities that may have an adverse impact on a woman's fertility to be diagnosed by a non- or minimally-invasive means. These abnormalities, once detected, could be treated accordingly prior to initiating ART treatments.

Irrespective of the stimulation regimen, a pre-treatment "baseline" ultrasound examination will ensure that the ovaries are "quiescent" and free from any large residual cyst that might hamper an adequate ovarian response. Identification of such cyst(s) or hydrosalpinges prior to treatment will be useful in differentiating them from developing follicles later on. A pre-treatment transvaginal ultrasound examination may also predict ovarian responsiveness to gonadotrophins in *in vitro* fertilization.

Monitoring ovarian response

Various methods have been employed to monitor the ovarian response to controlled hyperstimulation. Nonetheless, ultrasound evaluation has remain the mainstay for assessing the adequacy of ovarian response and the timing of hCG administration. As early as 1984, the Cromwell group has been able to achieve a comparable pregnancy rate by using ultrasound alone without the use of hormonal assays for the timing of hCG administration thereby simplifying the treatment procedure and reducing cost.

Ultrasound-guided oocyte retrieval

Since Lenz *et al* first described ultrasound-guided follicle aspiration in the *Lancet* in 1981, this approach has largely replaced laparoscopic retrieval. The procedure can be performed in an ambulatory setting with conscious sedation. Women with extensive pelvic adhesions with "inaccessible (to the laparoscope) ovaries" no longer pose a problem. The *transvaginal* route is predominant because of the close proximity of the ovaries to the vaginal fornices and the avoidance of puncturing the urinary bladder. In selected cases, e.g. when the ovaries are stuck to the uterine fundus or high on the pelvic side-walls, the *transabdominal* or *transurethral* route may provide better access and, therefore, preferred. The complication rate is low (< 2%). Serious complications such as pelvic abscesses and vaginal haematomas are uncommon although perforated appendicitis and fatality have been reported.

Evaluation of uterine receptivity

The success of ART depends on both the embryo quality and the uterine receptivity. Most investigators agree that the positive predictive value of endometrial thickness alone is low, but a thickness $\leq$ 6 mm is highly predictive of non-conception. In contrast, a multilayered echogenic endometrial pattern is said to be predictive of successful implantation. More recently, investigators have turned to the ultrasound indices of uterine blood flow as predictors of successful ART outcome. The results are again conflicting with some investigators demonstrating a significant difference in uterine blood flow impedance (pulsatility index -- PI) between conception and non-conception cycles and others finding no difference. Even when a significant difference can be demonstrated, there is often considerable overlap between the PI values in the two groups. Nevertheless, there appears to be a general agreement that inadequate uterine blood flow impaired implantation but optimum uterine perfusion may not necessarily lead to conception.

Management of complications associated with ART treatments

Ultrasonography has important applications in the prevention, diagnosis, monitoring and even treatment of several complications that arise from ART. One example is ovarian hyperstimulation syndrome (OHSS). Although serum E_2 on the day of hCG administration is a better predictor of OHSS, there are ultrasound indicators that have been shown to be useful. In established OHSS, ultrasonography can be helpful

in detecting associated ascites and monitoring the ovarian size. In rare instances, ultrasound is used to guide paracentesis in women with severe respiratory compromise due to OHSS.

ART procedures are associated with a 20 to 30% risk of multiple pregnancies. Recently ultrasonography, particularly the transvaginal approach, has been applied to selectively reduce higher order (quadruplets or above) multiple gestations.

Future developments

The application of sonohysterogaphy in the evaluation of women with repeated implantation failures despite good embryo quality may be further explored.

Two recent reports, one using colour Doppler imaging and pulsed Doppler spectral analysis and the other using power Doppler, have suggested that follicular vascularity may be an important parameter in the assessment of folliculogenesis and a possible predictor of outcome of ART. The major drawback of the application of colour Doppler imaging has been the inconsistent results obtained from different centres.

Finally, preliminary data has suggested that three-dimensional ultrasonography is more accurate in predicting follicular volumes particularly within the clinically useful range (3-7 ml) for IVF-ET. Further studies are required to determine if this technique might improve the outcome of ART.

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Laboratory Requirement and Protocol

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In any assisted reproductive technology (ART) program, laboratory services are a pre-requisite as they are needed:

1. to perform routine semen assessments and special tests to aid in making diagnosis about the cause of infertility for the couple,
2. to perform hormonal assays for cycle monitoring, e.g. oestradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, βhCG, progesterone and pregnancy test,
3. for manipulation of the gametes, *in vitro* fertilization, culturing of the embryo, as well as cryopreservation and storage of semen and embryo.

Although the first two objectives may not have to be incorporated into the ART program as many private laboratories or even the hospital itself will be able to provide these services, the last objective has to be performed in a specialized laboratory - the IVF laboratory. In fact, a good IVF laboratory is one of the key components for the success in any ART program.

The setting up of an IVF laboratory needs careful considerations. Discussions will be made on the location and design of the IVF laboratory, as well as the types of equipment essential for the functioning of the laboratory. The laboratory design and the equipment available will in a way affect the protocol of the operation. So every IVF laboratory should have a carefully written protocol with details on all the steps involved for every procedure carried out in the laboratory. This will aid in the training of laboratory personnel and provide assurance in maintaining quality service in the AR program.

The main activities in an IVF laboratory include:

1. Medium preparation
2. Oocyte identification during transvaginal ultrasound-guided oocyte retrieval and subsequent culture
3. Sperm preparation and insemination
4. Checking of fertilization status of the oocytes
5. Culture of embryos
6. Assessing the developmental status of the embryos and embryo transfer
7. Embryo cryopreservation and storage

8. Thawing of frozen embryos for replacement
9. Micromanipulation (ICSI) with ejaculated sperms or sperms obtained from either MESA (microsurgical epididymal sperm aspiration) or even TESE (testicular sperm extraction from testicular biopsies)
10. Semen or sperm cryopreservation
11. Sperm preparation for intrauterine insemination

The protocol for the various procedures will be discussed. Other procedures such as gametes intra-fallopian tube transfer (GIFT) and pronuclear stage (zygote) intra-fallopian tube transfer (PROST or ZIFT) are no longer carried out in our ART program.

Quality control and assurance are very important in the IVF laboratory. The IVF laboratory should be kept clean and tidy at all times, and activities carried out in the laboratory should be documented. Equipment should be properly maintained and regularly serviced.

Care should also be taken for chemicals, media and consumables used for culturing and handling of gametes. Any new batch of chemicals, medium and culture ware should be tested for possible presence of toxins before introduced into the AR program. All human materials should be considered potentially infectious, so disposable plastic ware is used for culture. When required, glassware and plastic disposables should be properly cleaned and sterilized before use. **All disposables involved in culturing and handling of gametes and embryos must be properly labelled with patient's name.**

Communication between clinicians and the IVF laboratory is also very important in an ART program. Any abnormalities or unusual findings during the treatment procedure should be discussed immediately; the clinicians, laboratory personnel and the patient involved should be well informed of any decision made. Regular meetings should be held between the clinicians in charge of the stimulation procedure, the nurses and the IVF laboratory personnel. Discussions are made on: (1) current cases so that the laboratory personnel could be well prepared prior to the operation; and (2) failure cases in order to find out the reason for the failure and plan to be taken in subsequent cycle of treatment. It is only through regular review and discussions can improvements be made in order to provide a quality service in an assisted reproduction program.

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Sperm assessment and sperm preparation

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The common methods used for sperm assessment are those based on World Health Organization. The normal values for semen variables according to WHO (1992) are as follow:

Volume	≥2.0 ml
pH	7.2-8.0
Sperm concentration	≥20 x 10^6 spermatozoa/ml
Total sperm count	≥40 x 10^6 spermatozoa/ejaculate
Motility (within 60 min)	≥50% forward progression or ≥25% rapid progression
Morphology	≥30% normal
Vitality	≥75% excluding dye
White blood cells	<1 x 10^6/ml

The protocols for these assessment can easily be obtained from the manual published by WHO (1992). In order to have valid information on the fertility of the individual, proper standardized procedures have to be used. Several points in collecting the semen samples have to be noted.

1. Never conclude the diagnosis from the assessment of a single semen sample, as semen parameters even from a normal male can vary considerably among different samples. Initial evaluation should be based on two samples, with interval of not less than seven days or more than 3 months apart. Additional samples should be collected if the results of former assessments are markedly different.
2. Semen samples should be produced by masturbation and collected in sterile bottle. If coitus interrupts is used, the sample may be contaminated with vaginal contents, or some spermatozoa may be lost. Condom should not be used as it may contain spermicide.
3. Patients should be asked to abstain from ejaculation for at least 2 days.
4. Sperm motility will decline significantly and viability of sperms from subnormal samples may decline after 3 h at room temperature. Therefore, semen sample should be delivered and examined within 2 hours.

While the predictive value of conventional semen analysis on fertilization is limited, semen assessment do permit some rational decisions to be made and provide some guidance on the management of the couple. For instance, the presence of multiple sperm defects is a useful predictive factor in intrauterine insemination. Apart from

these descriptive diagnostic andrology, other methods have been developed to assess specific function of the sperms. These include :

1. Hypo-osmotic test - This is used to determine the viability of the sperm. The method bases on the semi-permeability of the intact cell membrane, which causes viable spermatozoa to 'swell' under hypo-osmotic condition
2. Acrosome reaction with or without ionophore induction - This is to test the integrity of the acrosome membrane. Several agents can be used to stain the acrosome membrane. These include peanut agglutinin, antibody against inner acrosome membrane, and chlortetracycline.
3. Zona binding test - This is to test the capacity of the sperm to bind to zona pellucida. The commonly used method is the hemizona binding test. As sperm-zona binding is species specific, the availability of the human zona limits the widespread use of this test for general sperm assessment.
4. Computer assisted sperm analysis - This method utilizes computer imaging technique to measure the sperm motility objectively.
5. Morphology (strict criteria) - This method is similar to the WHO protocol for morphological assessment of sperm. It differs from the WHO criteria by considering all the sperm with marginal morphology as abnormal.
6. Zona free hamster egg penetration test - This is to test the ability of the sperm to fuse with the zona-free hamster egg and to decondense after fusion. It is based on the assumption that the human sperm-hamster egg fusion is the same as human sperm-human egg fusion. In order to reduce false negative result, calcium ionophore may be used to enhance sperm-oocyte fusion.

Although these new methods of examination are useful, they are not usually used for the analysis of semen in routine practice. This is because some of them require special techniques and trained personnel are necessary, while others require expensive equipment e.g. computer assisted sperm analysis system, or material of limited availability e.g. human zona pellucida.

Sperm assessment should aim at identifying whether a specific treatment is likely to be unsuccessful, and preventing inappropriate early intervention. These will provide the guidance for selection of the most appropriate form of treatment for each couple, especially those with male factor infertility.

In order to increase the success rate in assisted reproduction, semen samples are usually processed and the better sperms are harvested. There are two sperm preparation protocols commonly used in assisted reproduction program. They are the swim-up method and the density gradient centrifugation method. The former isolate sperm of good motility, while the latter purifies sperms with higher density. Reports have shown those sperms with higher specific gravity also possess better morphology and motility. These methods will be discussed. The density gradient centrifugation method is the most widely used method for sperm preparation.

Gamete and Embryo Cryopreservation

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The cryopreservation of semen sample or sperms for use in the assisted reproductive technologies (ART) program has been a common practice in many IVF centres. With the introduction of embryo cryopreservation and frozen-thawed embryo replacement into the ART program, the chance of pregnancy resulting from one cycle of oocyte retrieval and *in vitro* fertilization is improved.

The advantages of cryopreservation of semen sample are:

1. it enables the female to continue with the ART treatment when her male partner is away,
2. the frozen semen may serve as a backup and minimize the stress faced by the couple undergoing ART treatment, as some people are unable to submit the semen sample at a specific time,
3. semen cryopreservation and storage can be offered to patients who are at risk of losing their fertility capacity through chemotherapy or radiotherapy.

With the ICSI technique, only very few sperms are required to fertilize the oocytes in each cycle. So very often there will be surplus sperms collected from the MESA (microsurgical epididymal sperm aspiration) or TESE (testicular sperm extraction) procedure, and these sperms may be cryopreserved for use in subsequent cycle so that the husband need not go for another surgical operation.

Glycerol is the cryo-protectant used in semen cryopreservation. The preparation of the freezing solution and the protocol for semen cryopreservation in our program will be discussed.

Although freezing of sperm is well established, there is yet no established freezing protocol for oocyte cryopreservation.

A successful embryo cryopreservation program plays a very important role in the assisted reproductive technologies service, as it:

1. maximizes the use of good quality embryos,
2. minimizes the chance of multiple pregnancy and associated complications,
3. improves the chance of pregnancy from one oocyte retrieval and *in vitro* fertilization procedure through multiple embryo transfers,
4. enables embryo transfer to be postponed in patients who are at risk of developing ovarian hyperstimulation syndrome or other complications,

5. enables the oocyte/embryo donation program to be carried out.

In our IVF laboratory, embryo cryopreservation is usually performed on Day 2 embryos, i.e. on the day of embryo transfer, using propanediol (PROH) as the cryo-protectant. Cryopreserved embryos are stored in liquid nitrogen tank until time for replacement. Frozen-thawed embryo replacement can be performed in a natural cycle, a clomiphene-citrate induced cycle, or in an artificial cycle whereby the endometrium is prepared with exogenous steroids. The protocol for embryo cryopreservation and thawing of frozen embryo will be discussed.

Running an embryo cryopreservation program do have problems, these include:

1. an increasing demand on storage space needed for the frozen embryos,
2. what to do with unclaimed embryos, and
3. legal and ethical problems on the disposal of the embryos when the couple divorce or when one of the parties pass away.

This is the universal dilemma faced by all assisted reproductive technologies services.

Currently development on the freezing of ovarian cortical tissue and *in-vitro* maturation of preantral follicle are underway. In the future patients who are at risk of losing their ovarian function through pelvic disease, surgery, radiotherapy or chemotherapy may be able to have their fertility potential preserved.

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Oocyte and Embryo Donation

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Oocyte and embryo donation is a means by which an infertile woman, who cannot produce her own ova, can have a child with the help of IVF-ET. Since it is a serious matter to donate one's gametes to another. Both donor and recipient should be involved in the process. It would ever play a significant role in assisted reproduction.

Background

At present decade, with the sperm donation, the oocyte and embryo donation become an efficacy method of assisted reproductive techniques. The first successful embryo transfer was performed in the rabbit 100 years ago (Heape 1890); the first successful embryo transfer in the human was reported by Buster *et al* in 1983. With the successful development of *in vitro* fertilization (IVF) technology after 1980, it followed that donor oocytes could be fertilized *in vitro* and transferred to recipient endometria. The first pregnancy resulting from the transfer to a recipient of a donor oocyte fertilized *in vitro* was reported by the Monash IVF group (Trounson et al. 1983). In 1984, the same group reported the achievement of a pregnancy in a patient with primary ovarian failure (POF) using hormone replacement therapy (HRT). Van Steirteghem reported the first egg donation pregnancy from frozen-thawed embryo transfer, and cryopreservation has proved extremely important to the development of ovum donation programs.

Perhaps the greatest advance has been in simplification of the steroid replacement regimen, as described by Serhal and Craft and several regimens and modes of administration are now in use.

Indications for oocyte donation

Recipients

Patients requiring egg donation fall into two categories, firstly, patients without spontaneous menstrual cycles :

1. Premature ovarian failure: it is estimated that 1% of woman under a premature menopause (before the age of 40);
2. Gonadal dysgenesis, including Turner's syndrome;
3. Ovarian failure due to medical treatment (chemotherapy, radiotherapy) : this group is increasing in number as survivors of childhood cancers reach adulthood;
4. Surgical oophorectomy.

The second category are patients with spontaneous menstrual cycles :

1. IVF failure: cycling women with repetitive IVF failure due to poor ovarian response to superovulation make up a large proportion of patients requesting egg donation; there is also a group who have repetitive failure of fertilization due to oocyte abnormality;
2. Carriers of genetic disease: couples at risk of having children with fatal or severely disabling inherited disease may request gamete donation;
3. Carriers of chromosomal abnormalities: a small number of women suffer repeated pregnancy loss due to chromosome abnormality or are at high risk of a second Down's syndrome child.

Donors

Sources of donated eggs

1. Volunteers: parous "sterilized" women (using IUD or had ligation of the tubes) willing to undergo stimulation and oocyte recovery for donation to anonymous recipients for altruistic reasons.
2. Known donors: donations from relatives or friends of the recipient have also been practiced in our unit.
3. *In vitro* fertilization (IVF) donors: patients undergoing superovulation for IVF or gamete intrafallopian transfer (GIFT) may volunteer to donate excess eggs. However, with the availability of cryopreservation, most patients wish to have spare eggs fertilized and embryos stored for their future use. In China, because of the one child policy, when an infertile couple gets pregnancy, they would willingly donate their embryos to other couples.

Characteristics of egg donors

1. Age: the risk of chromosome abnormal pregnancies and of the genetic mother; hence egg donors should be under 35, and the value of antenatal diagnosis should be discussed with the recipient where appropriate.
2. Parity: we recommend that all donors should be parous, both for psychological reasons and also because success rates appear to be better using fertile donors.
3. Physical characteristics: the race, hair and eye colour, height, weight and built of the donor are recorded. These are matched as closely as possible to the characteristics of the intended recipient.
4. Blood group: the blood group of the donor is checked and matched to the recipient where appropriate (e.g. when the recipient couple is Rhesus negative).

Steroid Replacement Schedules

I. Incremental regimens

This regimen, designed to mimic the hormonal changes of a normal ovulatory cycle, uses incremental doses of oral oestradiol valerate with the introduction of progesterone pessaries from day 15 of the cycle. Pregnancy is then supported with oral oestradiol and intramuscular progesterone. (Figure 1)

Although this schedule of hormone replacement is physiological, its complexity can be a disadvantage. Moreover, there is only a limited period for transfer per cycle, giving little opportunity for synchronization.

II. Constant-dose

With a variable length 'follicular' phase, a constant supra-physiological dose of hormone replacement is administered (oestradiol valerate 2 mg tid with progesterone-in-oil 100 mg daily intramuscularly). Progesterone is started on the day before egg collection.

III. Monitoring the replacement cycle

Endocrine and histological responses by serial blood samples and a timed (day 21) endometrial biopsy. Good endometrial development is the critical 'end-point' of therapy. Treatment can then be adjusted if necessary before embryo transfer.

IV. Synchronization

A. *Recipient*

Agonadal recipients with artificial cycles can quite easily be aligned to the donors cycle. Regimens with a fixed dose, variable length of follicular phase are ideal for this purpose.

Recipients with natural cycles are more difficult to adjust. The recipients menstrual period can be delayed by norethisterone or the oral contraceptive pill given in the luteal phase of the preceding cycle.

A more reliable method is to use GnRH analogue to suppress the recipient cycle, followed by hormone replacement therapy identical to the regimen for agonadal women.

B. *Donor*

Manipulation of the donor cycle can be achieved in two ways :

1. Postponing the menstrual period.
2. GnRH-a stimulation protocol.

V. Pregnancy following egg donation

In spontaneous cycle, the corpus luteum of the ovary responds to stimulation by human chorionic gonadotrophin to maintain the pregnancy.

In HRT cycle, the maintenance of pregnancy by exogenous hormone replacement until the time of the "Luteo-placental shift" is critical. The ovarian hormones, oestrogen and progesterone are the only ones to allow early pregnancy development. As the trophoblast develops, it contributes to steroid hormone production and the "Luteo-placental shift" occurs around 7 weeks of pregnancy. The precise requirements of early pregnancy are not known, it depends on serum E_2 and P level, Oocyte donation programs mimic the rise in steroid levels of spontaneous pregnancy by increasing hormone replacement. On receipt of a positive pregnancy test, we double the steroid dosage of 8 mg estradiol valerate daily and 100 mg progesterone intramuscularly daily.

VI. Once the fetoplacental unit is active, it is safe to withdraw therapy and this is around 12 weeks of pregnancy.

VII. Outcome of pregnancy

A. Miscarriage

It is difficult to give an accurate figure for the percentage of pregnancy losses after egg donation treatment. Given adequate hormone replacement, the risk of early pregnancy loss would be expected to be equivalent to other assisted conception techniques. There is no evidence at present that agonadal patients, who usually have a very small or infantile uterus before treatment, are more likely to miscarry or labour prematurely.

B. Complications

It has been suggested that pre-eclampsia is common in egg donation pregnancies and that this may have an immunological basis. However, a high proportion of women conceiving with egg donation are primigravidae and in the later childbearing years, and both these factors are associated with toxaemia.

Ethical and legal issues

If treatment is successful, the child born will be genetically that of the donor, and will pass these genes to the next generations, so oocyte donation involves rather different considerations compared with other tissue donation. Egg donors must be highly motivated. All donations are anonymous. Donors are volunteers.

Alternative methods of conception

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In vitro fertilization and embryo transfer (*IVF-ET*) is by far the commonest method of assisted reproduction employed to treat various causes of subfertility in many units throughout the world. The increase in demand for infertility treatment, particularly IVF-ET and the need for repeated attempts have led to a strain both on the medical resources and also on the limited finances available for health care. Many alternative methods have emerged in an attempt to simplify and reduce the cost. Simplification is of value to patients, to the medical team and to society. The treatment success may also be improved.

The alternative techniques can be classified into two main groups depending on *in vivo* and *in vitro* fertilization. *In vivo* fertilization methods include homologous artificial insemination (*AIH*) with or without superovulation, gamete intrafallopian transfer (*GIFT*) and direct oocyte transfer (*DOT*). *In vitro* fertilization methods are variants of IVF or GIFT treatment and include pronuclear stage tubal transfer (*PROST*), zygote intrafallopian transfer (*ZIFT*) and tubal embryo transfer (*TET*).

Intrauterine insemination (*IUI*) is one of the commonest AIH methods and involves direct transfer of spermatozoa into the uterine cavity. The rationale of this treatment is to bring a large number of motile spermatozoa in a concentrated volume as close as possible to the site of fertilization at around the time of ovulation. The cervical barrier is bypassed and a larger number of motile spermatozoa reach the ampullary region of the Fallopian tube after the insemination procedure to increase the chance. Other insemination techniques such as direct intraperitoneal transfer, fallopian tube sperm transfer and intra-follicular transfer have been developed to improve the outcome. The roles of superovulation and of increasing the frequency of insemination will be discussed. The results of AIH for different indications will be compared to other assisted reproduction techniques.

The direct transfer of oocytes and washed sperm to the Fallopian tubes is generally known as GIFT and it is the single most significant and successful developments in assisted conception treatment since IVF. The advantages of GIFT over IVF are that the tubes are considered a more natural environment for the fertilization process and the early embryonic development to occur. The timing of the transport of the zygote along the tube will allow for a more natural chance of implantation to occur. It has been reported that GIFT is more successful than IVF although there is no conclusive evidence. The main limitations of GIFT are that one of the tubes must be normal and fertilization cannot be shown to have occurred prior to the transfer of gametes. The need of general anaesthesia and the wastage of supernumerary oocytes greatly

increase the cost of one treatment cycle. More recently, attempts have been made to simplify GIFT further by collecting oocytes by aspiration of follicles under transvaginal ultrasound guidance and transferring the gametes into the tubes transcervically rather than laparoscopically. It is now also possible to carry out laparoscopic transfer under local anaesthesia as an office procedure.

Pronuclear stage tubal transfer (*PROST*), zygote intrafallopian transfer (*ZIFT*) and tubal embryo transfer (*TET*) represent a modification of the GIFT procedure for couples in whom GIFT is possible but for whom evidence of fertilization is important before transfer such as male factor or unexplained subfertility. Direct oocyte transfer (*DOT*) was first described by Craft et al. in 1982 and involves the transfer of oocytes and sperm directly to the uterus. It is a simplification of the IVF technique but unfortunately the limited success of this procedure has prevented its widespread use.

The increasing demand for assisted reproduction by patients has led to the development of new or alternative methods and each new technique must be carefully evaluated and compared to the existing well established reproductive technologies.

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Medical Treatment of Male Infertility

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Male factors are present in nearly half the couples consulting for infertility. The approach to the management of male infertility includes careful history taking and examination for general and local causes of abnormal semen quality, hormone assays, semen analysis according to WHO recommendation with or without objective semen assessment by computer assisted analysis of video recordings. Causal factors must be treated whenever possible. Deleterious environmental factors and toxic medications should be eliminated. Remedial surgical causes should be corrected. Male accessory gland infection needs to be treated by adequate antibiotics, and long term treatment may be required to prevent re-infection. Tuberculosis infection remains are important cause for male infertility in this region. Immunological infertility is rare in Chinese. The use of high-dose corticosteroid treatment is associated with serious side-effects and is not justified by the poor therapeutic efficacy. Endocrine causes of infertility is usually accompanied by symptoms and signs of hypogonadism. Hypothalamic-pituitary causes include prolactinoma and other pituitary tumours. The poor semen quality may or may not recover spontaneously with the treatment of the primary disorder. In subjects with hypogonadotrophic hypogonadism, induction of spermatogenesis is possible with exogenous gonadotrophin injection or LHRH infusion, but response is poor if testicular volume is less than 8 ml. Attempts of hormonal stimulation of deficient spermatogenesis due to idiopathic causes with tamoxifen, human menopausal gonadotrophin and pure FSH had been tried but the overall result was unsatisfactory. Assisted reproductive technology should be offered in well-defined cases.

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Surgical Treatment of Male Infertility

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There are two main types of surgical treatment for male infertility:

1. to promote spermatogenesis and
2. to overcome obstruction

Surgery to promote spermatogenesis

The varicocoele controversy

The report by late Mr. Selby Tulloch of restoration of sperm output in an azoospermic man following varicocoele ligation set the trend for modern varicocoele surgery. Although varicocoele is often referred to as "the most common cause of correctable infertility", the scientific basis for this statement has been widely challenged.

After extensively reviewing the clinical literature since the 1954 article by Tulloch, the following conclusions were made:

1. Existing data are flawed because of inappropriate study design and reporting.
2. In spite of the occasional study which indicates that varicocoelectomy does not improve fertility, the preponderance of the literature does in fact support a favorable effect.
3. Varicocoelectomy does appear to have a beneficial effect on sperm density. This effect seems more pronounced when initial sperm densities are greater than 10 million/ml. Conversely, less of a response may occur when preoperative sperm densities are greater than 40 million/ml.
4. Motility and morphology may improve significantly after varicocoelectomy when an associated rise in density has occurred.

Surgical treatment of obstruction

Vasectomy Reversal

Collected results indicate that there may be a slight advantage for microsurgical techniques both in patency and pregnancy rates. However operative technique is not the major determining factor and other factors are more important such as the presence of secondary epididymal obstruction, the presence of antisperm antibodies

and the time interval between vasectomy and reversal. The falling success rate with time is in part because of secondary epididymal obstruction and in part because of antisperm antibodies.

Epididymo-vasotomy

The success of epididymo-vasotomy depends on the cause of the obstruction and the techniques used. In general the success is low and a realistic prognosis should be given to the couple before any treatment. The prognosis is much better following treatment of post-inflammatory obstruction because this usually affects the tail of the epididymis making a low anastomosis possible.

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The Use of Electroejaculation in the Treatment of Anejaculatory Males

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For 14 years the authors have used electro-ejaculation (EE) in the neurologically impaired male. Initial subjects were spinal cord injured (SCI). When EE proved an effective and safe technique for all levels of SCI we studied EE in other cases of anejaculation, mainly Multiple Sclerosis (MS), diabetes, post-RPLND (Retroperitoneal Lymph Node Dissection), Spina Bifida, and idiopathic causes. The equipment is specifically designed and built 120/220 V AC sine wave 50-60 Hz stimulator, electrically isolated and approved for patient use, connected to a rectal probe with built in temperature indicator with automatic shut off when a preset temperature is reached. We have used this equipment to obtain ejaculates from 420 SCI, 38 RPLND, 27 idiopathic, 17 diabetic, and 6 MS subjects. Ages (yrs) of SCI subjects av. 32.1; range 16-61; non-SCI av. 35.8; range 25-67. Over 4,000 EE procedures performed in our study to date with no adverse effects reported. Stimulation parameters for ejaculation, are av. 9 V and 200 mas; range 4 to 20 V and 100-700 mas. The ejaculate can be both antegrade and/or retrograde. For retrograde ejaculate, the bladder is emptied and flushed just prior to EE procedure. Unique factors of EE are : inexpensive, repeatable procedure, safe and effective. We expect 100% ejaculation in all subjects (all ejaculates may not necessarily be capable of fertilization). Six hours of physician training in the science of EE is suggested. The EE equipment developed by the authors is used in some 115 University Hospitals, Andrological and IVF Clinics, and Research Institutions worldwide. Successful pregnancies have been obtained with men having the above listed etiologies of anejaculation.

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Micromanipulation in ART

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Micromanipulation in assisted reproduction technology involves the use of micro-needles and micro-pipettes to manipulate human oocytes and embryos. Its basic setup include an inverted microscope, a pair of micromanipulators and a pair of injectors. The inclusion of warm stage or incubator in the system is certainly beneficial to the embryo during micromanipulation. During micromanipulation, usually one micro-needle and one micro-pipette are used. The micro-pipette is used for holding the oocyte or embryo during micromanipulation, while the micro-needle is for manipulating the sperm/oocyte/embryo. Laser beam has also been used to replace the micro-needle for some of the procedures during micromanipulation.

Micromanipulation is involved in three aspects of assisted reproduction, namely assisted fertilization, assisted hatching and preimplantation diagnosis. In recent years, most developments on micromanipulation techniques have been focused on assisted fertilization. This is the technique by which we increase the fertilization rate of patients with previous unsuccessful *in vitro* fertilization. There are a number of methods for assisted fertilization. They are:

1. **Zona drilling** - This method uses acid tyrode solution to drill a hole on the surface of the zona. This will allow motile sperm to pass through the hole and fertilize the oocyte.
2. **Partial zona dissection (PZD)** - This is similar to zona drilling. Instead of using acid solution, a micro-needle is used to create a slit on the surface of the zona for easier passage of the sperms into the perivitelline space.
3. **Subzonal sperm injection (SUZI)** - This involves the injection of several motile or immotile sperms into the perivitelline space of the oocyte. Hopefully one of them will fertilize the oocyte.
4. **Intracytoplasmic sperm injection (ICSI)** - This is the most commonly used method for assisted fertilization. It involves the injection of a single immobilized sperm into the cytoplasm of the oocyte.

Studies worldwide have shown that ICSI is the best method to be used for assisted reproduction. The advantages of ICSI include:

1. a high fertilization rate;
2. minimal number of sperms are required;
3. no polyspermic fertilization; and
4. result almost independent of semen parameters, e.g. motility.

As the requirement for sperm is so low, ICSI can be applied to almost all sperm samples including those aspirated from epididymis (micro-epididymal sperm aspiration; MESA) and testis (testicular sperm aspiration; TESA). The number of sperms obtained from these two latter type of samples is usually low, and the motility of the sperms is usually poor. Recently, this intracytoplasmic sperm injection technique has been applied to patients with testicular failure, in whom no matured sperm can be recovered. In these cases, spermatids, and more recently, secondary spermatocytes can be injected. Live births have been reported.

Assisted hatching is a technique by which a hole/slit is created on the zona pellucida of an embryo using micromanipulation. This procedure helps the embryo to hatch from the zona pellucida, and thus improves the subsequent implantation of the embryo. Both retrospective and prospective studies have demonstrated that assisted hatching was effective in embryos with zonae thickness of more than 15 microns. Apart from mechanically facilitating the hatching process, assisted hatching may improve embryo implantation by allowing earlier embryo-endometrium contact. Such early contact may enhance embryonic development potential and may optimize synchronization between embryo and endometrium, resulting in improved implantation efficiency. Assisted hatching has also been suggested to be useful in patients with inferior oocyte quality, older age patients with multiple implantation failures, patients with elevated basal FSH levels.

Preimplantation diagnosis is a technique by which one or two blastomeres of an embryo are removed (embryo biopsy or blastomere extraction) and their genotype determined. Embryo biopsy is usually performed at the 4-8 cell stage. The genotype of the extracted blastomere will be determined by fluorescent in-situ hybridization or polymerase chain reaction. Only embryo with the normal genotype will be replaced. As this diagnosis is expensive, it is used on patients with a high risk of transmitting the genetic defect to their offspring.

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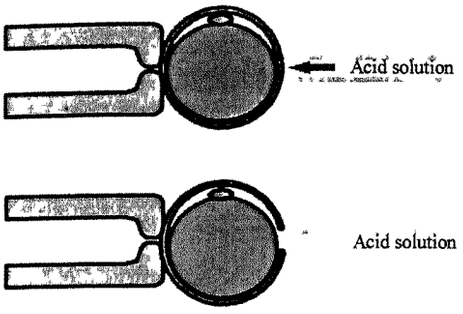
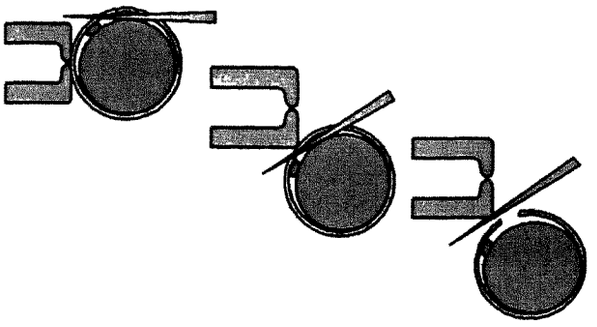
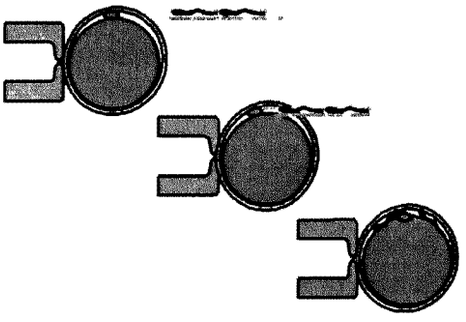
Zona Drilling



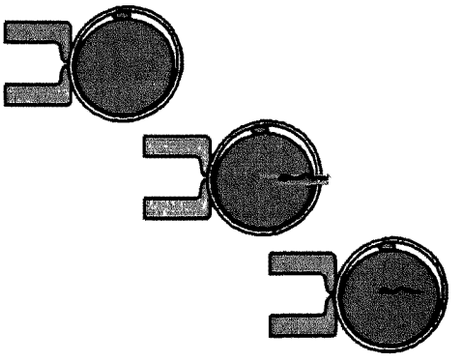
Partial Zona Dissection (PZD)



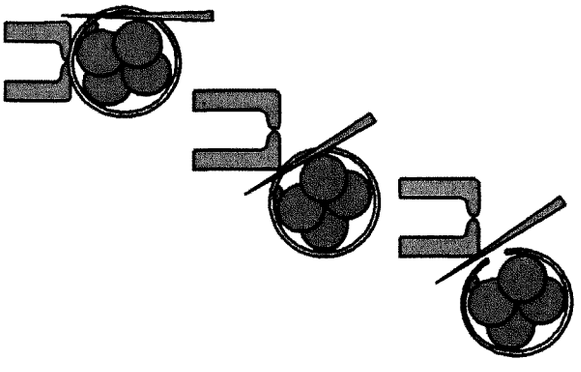
Subzonal Sperm Injection (SUZI)



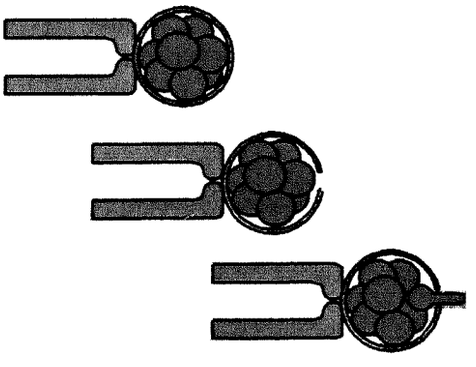
Intracytoplasmic Sperm Injection (ICSI)



Assisted Hatching



Blastomere Biopsy



Current and Future Advances in ART

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Ovarian Stimulation

The new preparations of recombinant follicle stimulating hormone (rFSH) is now available in some countries. It is almost 100% pure. The main advantages include: (a) it can be given subcutaneously, and (b) it is less likely to give rise to local reaction. There is some suggestive evidence that it is associated with a better pregnancy rate than human menopausal gonadotrophin (hMG) or urinary FSH.

The usual method of prevention of LH surge is the use of gonadotrophin-releasing hormone (GnRH) agonists. New preparations of GnRH antagonists are now available and they do not give rise to histaminergic side-effects. They are now being tested. Preliminary results suggest that a few doses of the GnRH antagonists are needed in the late follicular phase to prevent the occurrence of LH surge. A possible advantage is that the dosage of hMG required is less than that in cycles down-regulated with GnRH agonists.

Co-culture and Assisted Hatching

One of the problems of assisted reproduction is the low implantation rate of embryos. A number of studies have suggested that the use of co-culture may improve the success rates of *in vitro* fertilization (IVF) programs. However, the use of a co-culture system makes the program more complicated. There are also the problems of potential transmission of infection. Therefore, it is not used as a routine in most IVF programs. It is necessary to identify and isolate or purify the embryotrophic factors. Assisted hatching using a number of methods has also been shown to be effective in improving the pregnancy rates in patients with repeated failures of IVF. However, there is no evidence that the routine use of assisted hatching in all patients can improve the overall success rates. There is an increasing number of studies on implantation. We may be able to understand implantation better and improve our success rates.

Treatment of Male Infertility

The introduction of intracytoplasmic sperm injection has revolutionized the treatment of male infertility. Many couples with obstructive or non-obstructive azoospermia are now amenable to treatment. There is also better understanding of the genetic aspects of male infertility.

Pre-implantation Diagnosis

With the development of micromanipulation techniques, it is possible to take a biopsy of the embryos before transfer for genetic diagnosis using molecular biology techniques. It is also possible to determine the sex of the embryo to prevent the occurrence of sex-linked genetic diseases.

Cryopreservation of Oocytes

The technique of cryopreservation of mature oocytes is still not well developed enough for clinical application. There is an increasing number of reports suggesting that the cryopreservation of immature oocytes may be more likely to be successful. There is also the possibility of cryopreservation of ovarian tissue for subsequent auto-transplant. However, the report that cancers may be transmitted through the implantation of the ovarian tissue has caused concern for the safety of this procedure in preservation of fertility in female cancer patients.

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The Role of the Nurse in Assisted Reproduction Technologies

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Nursing is a professional practice and an academic discipline. It is the study and practice of caring in the human health experience. Using a knowledge base derived from theory, research and practice in biological, behavioural and nursing science, nurses diagnose and treat human responses to actual, or potential, health problems in order to promote, maintain and/or restore health.

Infertility is a state characterized by biological, psychological, social, spiritual and cultural dimensions. Over the past twenty years, knowledge of the diagnosis and treatment of infertility has advanced substantially while knowledge of its psychosocial, spiritual and cultural dimensions have lagged well behind. This paper describes the current state of knowledge of the multi-dimensional human responses to infertility and to the investigation and treatment associated with it. It demonstrates how nurses use this knowledge, in conjunction with knowledge acquired from biomedical research, to generate holistic models of care which can be applied in practice to promote the health and well-being of couples dealing with the crisis of infertility. It outlines the strengths and limitations of our existing knowledge of the multi-dimensional aspects of infertility and it argues for a multi-disciplinary, collaborative, and client-centered approach to infertility research and practice which will increase our understanding of our clients and improve the quality of care we provide.

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▼ **GONAL-F®** recombinant human Follicle Stimulating Hormone (follitropin alpha) (for subcutaneous / intramuscular injection) **Presentation:** Ampoules containing either 75 IU or 150 IU recombinant human Follicle Stimulating Hormone (FSH). **Indication:** GONAL-F is indicated for: (i) Anovulation (including polycystic ovarian disease, PCOD) in women who are unresponsive to clomiphene citrate. (ii) The stimulation of multifollicular development in patients undergoing superovulation for assisted reproductive technologies (ART) such as in-vitro fertilisation (IVF), gamete intra-fallopian transfer (GIFT) and zygote intra-fallopian transfer (ZIFT). **Dosage and administration:** To be administered by subcutaneous / intramuscular injection. Reconstitute immediately prior to use. Up to 3 ampoules of GONAL-F may be dissolved in 1 ml of diluent. Women with anovulation (including PCOD): The object of GONAL-F therapy is to develop a single follicle from which the ovum is liberated after hCG administration. GONAL-F may be given as a course of daily injections. In menstruating patients, treatment should commence within the first 7 days of the menstrual cycle. Treatment should be tailored to response, as assessed by (i) ultrasound and / or (ii) oestrogen secretion. A common regimen starts at 75 - 150 IU FSH daily, increasing by 37.5 IU or 75 IU, at intervals of 7 or 14 days. Abandon the cycle if response is inadequate. After an optimal response, a single injection of between 5 000 IU - 10 000 IU hCG is given 24 - 48 hours after the last GONAL-F injection. The patient is recommended to have coitus on the day of, and the day after, hCG administration. If response is excessive, treatment should be stopped and hCG withheld (see Precautions). Treatment recommences in the next cycle at a lower dose. Women undergoing ovarian stimulation prior to in vitro fertilization or other assisted reproduction technique: Down regulation with a gonadotrophin-releasing hormone (GnRH) agonist is commonly used to suppress the endogenous LH surge and control tonic LH levels. GONAL-F treatment typically starts 2 weeks after the start of agonist treatment. Both are continued until an adequate follicular response is achieved. A common superovulation regimen involves administration of 150 - 225 IU GONAL-F daily, starting on days 2 or 3 of the cycle. The dose is adjusted according to patient's response. After an adequate response, up to 10 000 IU human Chorionic Gonadotrophin (hCG) is given 24 - 48 hours after the last GONAL-F injection. **Contraindications:** Pregnancy, ovarian enlargement or cyst not due to polycystic ovarian disease (PCOD), gynaecological haemorrhages, ovarian, uterine or mammary carcinoma, hypothalamic or pituitary tumours, prior hypersensitivity to FSH, or when an effective response cannot be obtained. **Precautions:** Ovarian hyperstimulation syndrome (OHSS) can develop but is minimised by careful monitoring and withholding hCG; for superovulation, aspirate all follicles prior to ovulation. Self-administration of GONAL-F should only be performed by patients adequately trained and with access to expert advice. **Side effects:** Injection site reactions may occur. The possibility of OHSS exists; first symptoms are lower abdominal pain,possibly with nausea, vomiting and weight gain. In rare cases, OHSS causes fluid accumulation in the abdomen and thorax, as well as more serious thromboembolic complications. If ovarian response is excessive, GONAL-F treatment should be discontinued. The hCG injection should not be given. Abandoning treatment will reduce the chance of OHSS. Rarely, arterial thromboembolisms have been associated with menotrophin / hCG therapy. This may also occur with GONAL-F. Pregnancy loss is comparable with rates in women with other fertility problems. Ectopic pregnancy may occur in women with prior tubal disease. **Pharmaceutical precautions:** Store at or below 25°C, protected from light. **Legal category:** POM. **Pack size:** Ampoules of GONAL-F are packed singly and in boxes of 3 and 10 ampoules. Each ampoule is accompanied by an ampoule of 1 ml of water for injection. **Product Registration numbers for GONAL-F 75 IU and 150 IU:** HK-41249 and HK-41250 respectively.

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